PATENT COOPERATION TREATY



# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| 11006-98 | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/CA00/00650 | 02/06/2000 | 03/06/1999 |
| International Patent Classification (IPC) or national classification and IPC<br>C12N5/00 | | |
| Applicant<br>NATIONAL RESEARCH COUNCIL OF CANADA et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 12/12/2000 | 20.09.2001 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Loubradou-Bourges, N<br>Telephone No. +49 89 2399 7342 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00650

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
**Description, pages:**

| | |
|---|---|
| 1-36 | as originally filed |

**Claims, No.:**

| | | | | |
|---|---|---|---|---|
| 1-55 | as received on | 06/09/2001 | with letter of | 06/09/2001 |

**Drawings, sheets:**

| | |
|---|---|
| 1/13-13/13 | as originally filed |

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   **see separate sheet**

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 6, 8, 12, 14-31, 34-55 |
| | No: | Claims | 1-5, 7, 9-11, 13, 32-33 |
| Inventive step (IS) | Yes: | Claims | 52-55 |
| | No: | Claims | 1-51 |

| | | | |
|---|---|---|---|
| Industrial applicability (IA) | Yes: | Claims | 1-55 |
| | No: | Claims | |

2. Citations and explanations
**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
**see separate sheet**

Reference is made to the following documents:

D1: US-A-5 518 915 (NAUGHTON ET AL.) 21 May 1996 (1996-05-21)
D2: NYGAARD SVEIN J T ET AL: 'Dynamic determination of human glioma invasion in vitro.' JOURNAL OF NEUROSURGERY, vol. 89, no. 3, 1998, pages 441-447, XP000971892 ISSN: 0022-3085
D3: Steinstrasser I. et al.: "Physical model relating diffusional transport and concurrent metabolism of peptides in metabolically active cell sheets" J. Pharm Sci, 1995 Nov; vol 84 n°11, p. 1332-41
D4: US-A-5 314 805 (HAUGLAND ET AL.) 24 May 1994 (1994-05-24)

The document D3 was not cited in the international search report. A copy of the document is appended hereto.

## SECTION I

The amendments filed with the letter of 06.09.01 are considered to meet the requirements of Art. 34(2) b PCT.

## SECTION IV

The present application lacks unity *a priori* for the following reasons:
Claims 1-51 (invention 1) relate to a *in vitro* model of a mammalian tissue, comprising 3-dimensional aggregates of living mammalian cells.
Claims 52-55 (invention 2) relate to a method for predicting biological characteristic of a mammalian tissue.
The IPEA is of the opinion that these two groups of claims are not so linked as to form a single general inventive concept as there is no special technical feature in the sense of Rule 13.2 PCT linking together these two groups of claims, which therefore are considered to be separate inventions
According to Rule 68(1) PCT, the IPEA chose not to invite the applicant to restrict or pay additional fees.

## SECTION V

## 1. NOVELTY

The present application does not meet the requirements of Art. 33(2) and (3) PCT:

D2 relates to a study wherein brain tumor tissue is grown in a three-dimensional culture. The tumor cells are cocultured with fetal rat brain cells aggregates. The cells are stained with fluorescent dyes before the coculture.
The cells of the two different phenotypes are considered to be in a "predetermined initial proportion", since equally sized tumor spheroids and brain cell aggregates are used for incubation in the coculture, thus leading to predefined and constant initial proportion of tumor and brain cells (see p.442, Material and Methods, Fluorescent Dye Staining Procedures).
The "proliferation kinetics of the two types of cells" is considered to be "simultaneously assessed" since the tumor proliferation rate and the brain cell aggregates destruction are simultaneously measured and plotted (see p.445, fig.7).
Therefore, D2 is prejudicial to the novelty and the inventive activity of claims 1-5, 7, 9-11, 13, 32- 33.

## 2. INVENTIVE ACTIVITY

Invention 1: The present application relating to invention 1 does not meet the requirements of Art. 33(3) PCT:

2.1 The additional features of dependent claims 6, 8, 12, 14-15, 20-24, 27-30, 34-42 appear to be merely obvious alternatives for a skilled person in the art, and thus are not suitable to establish the presence of an inventive activity in said claims. The teaching of said claims relates to the use of specific fluorescent dyes, the specie and the phenotype of the cells used, etc. All these teachings are anticipated in D1 and D4 for example, which are documents from the same fields, namely three-dimensional culture systems and cell viability assays using dyes.

2.2 The use of three-dimensional systems for screening compounds for cytotoxicity, drug action, diagnosing and monitoring cancer is well known and described in the prior art.

For example, D1 (see p. 16 and 32) discloses a three-dimensional model system for the blood-brain barrier comprising tight junctional complexes, which may be used to test the ability of substances to cross the barrier. D1 also states that the three-dimensional systems are useful to study functional and morphological interactions since these systems are mimetic to the physiologic histology and present the cell-cell and cell-matrix interactions. D1 provides complete teaching for such screening.

D2, which is considered to represent the closest prior art to the subject-matter of claims 43-48 and 49-51, gives a clear incentive to use the disclosed three-dimensional tissue system as an experimental too for studying the effects of drugs on said system (see p.445, right column) and for evaluating therapy regimens directed against normal brain destruction as well as against biological parameters governing cell migration (see p.446, left column, last §).

Thus, in the light of D2 in combination with the teachings of D1 and the general knowledge of the skilled person in the art, the subject- matter of claims 43-48 and 49-51 which relates to method of screening of antitumour substance and of substance modulating gap junction intercellular interaction does not involve an inventive activity.

Invention 2:

The subject-matter of claims 52-55 appears to be novel and inventive (Art. 33(2) and (3) PCT in view of the available document D3, cited by the examiner for the following reasons :

D3 relates to a method wherein metabolically active cell sheets representing for example the epidermis are provided, parameters like substrate concentration gradients are generated by mathematical simulation, parameters are subjected to variation and thus, metabolism control and diffusion control are predicted.

However, D3 fails to provide or suggest a cellular automaton simulation model.

Thus, the method of claim 52 and dependent claims thereof is clearly distinguishable and inventive over the mathematical model described in D3.

**SECTION VIII**

The present application does not meet the requirements of Art. 6 PCT:

The term "SIMCEL-2D", "SIMCEL-3D" (claim 53) are internal designation, meaningless to a skilled person in the art.

ART 34 AMD

WHAT IS CLAIMED IS:

1. An *in vitro* model of a mammalian tissue, said model comprising 3-dimensional aggregates of living mammalian cells of at least two different phenotypes and a liquid growth medium.

2. A model according to claim 1, wherein the 3-dimensional cell aggregates are suspended in the growth medium.

3. A model according to claim 2, wherein the 3-dimensional cell aggregates are of an essentially spheroidal shape.

4. A model according to claim 3, wherein the 3-dimensional cell aggregates are formed in the absence of a solid support.

5. A model according to claim 3, wherein the 3-dimensional cell aggregates are formed in the presence of a solid support.

6. A model according to claim 5, wherein the solid support consists of porous beads.

7. A model according to claim 1, wherein the cells of at least one phenotype are fluorescently labeled prior to forming the 3-dimensional aggregates.

8. A model according to claim 7, wherein the cells are labeled with a fluorescent membrane linker.

9. A model according to claim 7, wherein the cells are labeled by loading with a fluorescent dye.

10. A model according to claim 1, wherein the 3-dimensional aggregates comprise cells of a first and of a second phenotype.

11. A model according to claim 10, wherein the cells of at least one phenotype are fluorescently labeled prior to forming the 3-dimensional aggregates.

12. A model according to claim 11, wherein the cells are labeled with a fluorescent membrane linker.

13. A model according to claim 11, wherein the cells are labeled by loading with a fluorescent dye.

14. A model according to claim 13, wherein the dye is calcein-AM.

15. A model according to claim 12, wherein the cells of the first and the second phenotype are labeled with fluorescent membrane linkers fluorescing at different wavelengths.

16. A model according to claim 10, wherein the cells of the first and the second phenotype are of human origin.

17. A model according to claim 16, wherein the cells of the first phenotype are normal cells of human origin.

18. A model according to claim 17, wherein the cells of the first phenotype are endothelial cells.

19. A model according to claim 18, wherein the cells of the second phenotype are tumour cells.

20. A model according to claim 19, wherein the endothelial cells are fluorescently labeled.

21. A model according to claim 20, wherein the endothelial cells are labeled with a fluorescent membrane linker.

22. A model according to claim 21, wherein the 3-dimensional cell aggregates are formed in the absence of a solid support.

23. A model according to claim 22, wherein the 3-dimensional cell aggregates are formed by covering particles of a solid support with a layer of the endothelial cells and seeding the tumour organoids to the layer of endothelial cells.

24. A model according to claim 23, wherein the solid support is capable of releasing a blood substitute.

25. A model according to claim 17, wherein the cells of the first phenotype are stromal cells.

26. A model according to claim 25, wherein the cells of the second phenotype are tumour cells matching the source of the stromal cells.

27. A model according to claim 26, wherein the cells of both phenotypes are fluorescently labeled with labels fluorescing at different wavelengths.

28. A model according to claim 27, wherein the labels are fluorescent membrane linkers.

29. A model according to claim 28, wherein 3-dimensional cell aggregates are formed in the absence of a solid support.

30. A model according to claim 28, wherein 3-dimensional aggregates are formed by growing a layer of the stromal cells on particles of a solid support and then growing a layer of the tumour cells on the layer of the stromal cells.

31. A model according to claim 17, wherein the cells of the second phenotype are tumour cells.

32. A model according to claim 13, wherein the cells of the first phenotype are cells of a tissue in which metastases of the tumour are expected to develop.

33. A model according to claim 32, wherein the cells of the first phenotype are epithelial cells.

34. A model according to claim 31, wherein the cells of the first phenotype are grown as a monolayer on one side of a porous solid support.

35. A model according to claim 34, wherein the tumour cells grown in the form of 3-dimensional aggregates are applied to the opposite side of the support.

36. A model according to claim 35, wherein the tumour cells are fluoroscently labeled.

37. A model according to claim 17, wherein the cells of the second phenotype are cells of the first phenotype treated with a chemical agent prior to forming the 3-dimensional aggregates.

38. A model according to claim 37, wherein the chemical agent is capable of blocking the proliferation of cells without killing the cells.

39. A model according to claim 38, wherein the chemical agent is mitomycin.

40. A model according to claim 37, wherein the chemical agent is a phototoxic agent.

41. A model according to claim 40, wherein the chemical agent is chloromethyl eosine diacetate.

42. A model according to claim 40, wherein the 3-dimensional aggregates of cells are illuminated with a light source after formation.

43. A method of screening for an antitumour substance, said method comprising the steps of:

a. providing a 3-dimentional *in vitro* model of human tissue according to claim 1, said model comprising at least one phenotype of tumour cells;
b. providing a candidate antitumour substance;
c. culturing the 3-dimensional cell aggregates for a predetermined period of time, in the presence and in the absence of the candidate antitumour substance;
d. measuring the cell proliferation rate of at least one cell phenotype in the 3-dimensional cell aggregates in the absence and in the presence of the candidate antitumour substance; and
e. accepting or rejecting the candidate antitumour substance based on results of the measurements of step d.

44. A method according to claim 43, wherein cells of at least one cell phenotype are fluorescently labeled.

45. A method according to claim 44, wherein cells are labeled with a fluorescent membrane linker.

46. A method according to claim 45, further including the step of dispersing the cell aggregates into a suspension of individual cells prior to measuring the cell proliferation rate.

47. A method according to claim 46, wherein the proliferation rate is expressed as the proliferation index.

41. A model according to claim 40, wherein the chemical agent is chloromethyl eosine diacetate.

42. A model according to claim 40, wherein the 3-dimensional aggregates of cells are illuminated with a light source after formation.

43. A method of screening for an antitumour substance, said method comprising the steps of:

a. providing a 3-dimentional *in vitro* model of human tissue according to claim 1, said model comprising at least one phenotype of tumour cells;
b. providing a candidate antitumour substance;
c. culturing the 3-dimensional cell aggregates for a predetermined period of time, in the presence and in the absence of the candidate antitumour substance;
d. measuring the cell proliferation rate of at least one cell phenotype in the 3-dimensional cell aggregates in the absence and in the presence of the candidate antitumour substance; and
e. accepting or rejecting the candidate antitumour substance based on results of the measurements of step d.

44. A method according to claim 43, wherein cells of at least one cell phenotype are fluorescently labeled.

45. A method according to claim 44, wherein cells are labeled with a fluorescent membrane linker.

46. A method according to claim 45, further including the step of dispersing the cell aggregates into a suspension of individual cells prior to measuring the cell proliferation rate.

47. A method according to claim 46, wherein the proliferation rate is expressed as the proliferation index.

48. A method according to claim 47, wherein the proliferation index is calculated from flow cytometry analysis of the cell suspension.

49. A method of screening for a substance modulating gap junction intercellular communication, said method comprising the steps of:

a. providing a 3-dimensional *in vitro* model of human tissue according to claim 1, said model comprising at least cell phenotype loaded with a fluorescent dye impermeant to the cell membrane;

b. providing a candidate modulating substance;

c. culturing the 3-dimensional cell aggregates for a predetermined period of time, in the presence and in the absence of the candidate substance;

d. measuring the migration of the dye to at least one other cell phenotype in the 3-dimensional cell aggregates, in the absence and in the presence of the candidate antitumour substance; and

e. accepting or rejecting the candidate modulating substance based on results of the measurements of step d.

50. A method according to claim 49, further including the step of dispersing the cell aggregates into a suspension of individual cells prior to measuring the migration of the dye.

51. A method according to claim 50, wherein the dye is calcein-AM.

52. A method for predicting a biological characteristic of a mammalian tissue, said method comprising the steps of:

a. providing a simulation model of the mammalian tissue;

b. setting model parameters;

c. running the model; and

d. evaluating the biological characteristic of the tissue based on results of step c.

53. A method according to claim 51, wherein the simulation model is SIMCEL-2D or SIMCEL-3D simulation model.

54. A method according to claim 52, wherein the biological response is the cyclic cell recruitment from the resting pool, the cell proliferation index, or intercellular diffusion.

55. A method according to claim 53, wherein the model parameters are set to simulate effects of physical or chemical agents on the tissue.